

Fisheries Biotechnology

INTRODUCTION: **Biotechnology** is also used in the fisheries field for increasing fish production through various techniques. Fisheries biotechnology can be broadly classified into aquaculture biotechnology, marine biotechnology, algal biotechnology and processing biotechnology.

Since 1980s, there has been a burst of biotechnology activity in research and development related to various **fish species**, in particular those used in aquaculture production. Biotechnology has played a major role in the areas of induction and control of

maturation and spawning, sex control (andro **gene** sis and gynogenesis), sex inversion in protandrous species like sea bass and protogynous species like the grouper, production of triploid, tetraploid and **transgenic** fishes. Traits that are being tested in fish species such as **carp, trout, salmon and channel catfish** include growth rates that are three to eleven times faster with more **efficient feed utilisation, increased tolerance to cold water and improved disease resistance**. Accelerated growth rates mean that fish reach marketable size sooner, thereby reducing overhead costs for fish farmers. In addition, researchers use the human interferon gene to improve disease resistance in carp, which could reduce the amount of **antibiotic** s needed to keep fish healthy and reduce the costs incurred from losses due to disease.

The first (and to date only) genetically engineered fish to be sold commercially is the fluorescent Glofish®, a zebra fish modified to glow red, which came onto the US market in 2004.

Other areas include disease diagnosis (molecular and immunodiagnostic kits), **hybridoma** technology, and management (probiotics, vaccines, immunostimulants), cell and **tissue culture** , conservation of germplasm (cryopreservation of fish gametes), extraction of bioactive substances from marine organisms including marine bacteria, marine algae, marine invertebrates and fishes.

TRANSGENIC FISH AND GENE TRANSFER TECHNOLOGY:

Introduction

An organism that has a foreign or modified **gene transfer** red to its **genome** using the **in vitro gene** tic techniques is called a genetically modified organism (GMO) or a **transgenic** organism.

- Gordon *et al.* (1980) produced transgenic animals by **microinjection** of **clone** d DNA into the pronucleus of fertilized eggs at the one-cell stage.
- Palmiter *et al.* (1982) introduced **growth hormone** gene into mice and produced giant mouse of 44 gms whereas, normal grows upto only 29 g.
- Attempts to produce transgenic fish began in the mid-1980s. Maclean and Talwar (1984) reported microinjection of cloned DNA into rainbow trout (*Oncorhynchus mykiss*) eggs.

Zhu *et al.* (1985) microinjected fertilized eggs of goldfish with metallothionein promoter fused with the human growth **hormone** gene.

Transgenic technology has been successfully used to develop fast-growing super-fish stocks for

- human consumption,
- to produce pharmaceuticals,
- to test water contamination in both developed and developing countries.
- Several laboratories now have GM fish with increased growth performance caused by extra copies of GH genes. So far, fast growing fish by transferring growth hormone gene have been developed for several aquacultural species.

Several species including loach, common carp, crucian carp, Atlantic salmon, channel catfish, tilapia, medaka and northern pike containing either human, bovine, or salmonid growth hormone genes grew 10-80% faster than non-transgenic fish in aquaculture conditions.

PCR amplification

It is based on repeated cycles of denaturation, annealing of **oligonucleotide primer** s complementary to the gene, and primer extension by Taq **polymerase** . The amplified fragment can then be recognized as a discrete fragment on a gel or on a southern blot.

HYBRIDOMA TECHNOLOGY

Our knowledge of the immune system of fish and fish diseases is extremely limited when compared to our knowledge of large animals. At present, fish farming (aquaculture) is becoming an increasingly important food production industry, and may play a significant role as a food source in the future. For this reason, application of the latest biotechnological advances, including MABs, to the aquaculture industry, is extremely important. MABs are being adopted for purposes of immunoassay and immunotherapy.

Hybridoma technology is a technology of forming hybrid cell lines (called **hybridomas**) by fusing a specific **antibody -producing B cell with a myeloma** (B cell cancer) cell that is selected for its ability to grow in **tissue culture** . The antibodies produced by the hybridoma are all of a single specificity and are therefore monoclonal antibodies (in contrast to polyclonal antibodies).

Hybridoma technology for the production of monoclonal antibodies (MABs) has contributed significantly to aquaculture. Monoclonal antibodies are being employed in disease, pathogen classification, epidemiological analysis and development of vaccines.

The idea of a " magic bullet " was first proposed by Paul Ehrlich who at the beginning of the 20th century postulated that if a compound could be made that selectively targeted a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity. In the 1970s the B-cell cancer multiple myeloma was known, and it was understood that these cancerous B-cells all produce a single type of antibody. This was used to study the structure of antibodies, but it was not yet possible to produce identical antibodies specific to a given antigen .

Production of monoclonal antibodies involving human–mouse hybrid cells was described by Jerrold Schwaber in 1973. The invention was conceived by Prof. Pieczenik, with Prof. John Sedat, as a witness and reduced to practice by Cotton and Milstein, and then by Kohler and Milstein.

Georges Köhler , César Milstein , and Niels Kaj Jerne in 1975; who shared the Nobel Prize in Physiology or Medicine in 1984 for the discovery. The key idea was to use a line of myeloma cells that had lost their ability to secrete antibodies, come up with a technique to fuse these cells with healthy antibody-producing B-cells, and be able to select for the successfully fused cells.

DNA Fingerprinting

DNA Fingerprinting technique can be used as a powerful **marker** system in identification in fisheries.

- i) Used to verify the identity of cultured cell lines and various lines of clonal fishes, including those obtained by gyno **gene** sis and androgenesis.
- ii) Useful as tools in demographic analysis of fish population. Their parents can be identified. i.e., identification of individuals and pedigree.
- iii) The fragments detected by DNA fingerprinting can also be used in **gene linkage analysis**. If a commercially important gene tightly linked to a fingerprinting marker, the transmission of the gene can be determined by inspection of the marker. This will be of great value in genetic improvement of fish.
- iv) Fish pathogens can be identified. .
- v) Individual specific pattern have been observed in rainbow trout (*O. mykiss*), Atlantic salmon, chum salmon (*O. keta*), coho salmon (*O. kisutch*) with M13 phage or **probe** s.
- vi) Useful in annexing paternal genetic contribution in gynogenetic fish.
- vii) Assessment of inbreeding rates,
- viii) to study the action of specific genes,
- ix) as genetic markers to identify individuals and family groups and the **labelling** of broodstocks to secure ownership property.

Applications of PCR

- Amplification of small amounts of DNA for further analysis by DNA fingerprinting.
- The analysis of ancient DNA from fossils.
- Mapping the human (and other species) genome.
- The isolation of a particular gene of interest from a tissue sample.
- Generation of probes: large amount of probes can be synthesized by this technique.

- Analysis of mutations: Deletions and insertions in a gene can be detected by differences in size of amplified product.
- Diagnosis of monogenic diseases (single gene disorders)
- Detection of microorganisms: Especially of organisms and viruses that are difficult to culture or take long time to culture or dangerous to culture.
- The PCR has even made it possible to analyze DNA from microscope slides of tissue preserved years before.
- Detection of microbial genes responsible for some aspect of pathogenesis or antibiotic resistance.
- Crucial forensic evidence may often be present in very small quantities, e.g. one human hair, body fluid stain (blood, saliva, semen). PCR can generate sufficient DNA from a single cell.
- The sensitivity of PCR allows the detection of pathogens that would be difficult to identify with conventional techniques.
- PCR is widely used for screening shrimp seed and brood for serious viral pathogens such as WSSV, YHV, IHHNV and TSV.
- PCR is ideal for studies in epidemiology, genotyping, health certification, quarantine and for screening for development of SPF stocks.